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CLOVER ANTHRACNOSE CAUSED BY COLLETOTRICHUM TRIFOLII

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JOHN MONTEITH, JR.

Associate Pathologist, Office of Vegetable and Forage Diseases Bureau of Plant Industry

UNITED STATES DEPARTMENT OF AGRICULTURE WASHINGTON, D. C.

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INTRODUCTION

The inability of red clover to maintain its relative position of prominence in the American farming system has been generally attributed to the frequent recurrence of what has been indefinitely termed "clover failure." It is well recognized that several factors separately or collectively may cause failure of a clover crop (*17*).[2] Diseases unquestionably contribute largely to these losses, and under certain conditions they are the sole agents responsible for destruction of the crop. In the United States the most important disease of clover thus far reported is anthracnose. There are, however, several

[1] This study was begun at the suggestion of A. J. Pieters, of the Office of Forage Crops, and much of it, especially the field phases, was done in cooperation with him. The author wishes to express his appreciation of Doctor Pieters's interest and aid throughout the progress of the work. The greenhouse and laboratory studies were conducted in cooperation with the department of plant pathology, University of Wisconsin.

[2] Reference is made by number (italic) to "Literature cited," p. 25.

fungi described as causing clover anthracnose, chief of which are *Colletotrichum trifolii* Bain and Essary (*5*) and *Gloeosporium caulivorum* Kirchner (*11*).

The scattered literature on this subject and reports received by the Plant Disease Survey of the Bureau of Plant Industry, indicate that these two fungi are frequently confused or at times regarded as identical (*8*, *10*, *23*). An investigation undertaken for the purpose of defining somewhat more clearly the rôle played by plant disease in clover failure led at once to a study of the relationship between these two important anthracnose-producing fungi. The phases which appeared to be of primary importance were those dealing with the influence of environmental factors on the distribution and severity of these diseases and the relative susceptibility of clovers of different origin to their attacks. It was soon evident that the two fungi, although producing very similar disease symptoms, were entirely distinct species—a distinction not founded merely on variations due to environment affecting spore characters or occurrence of setae. It is the purpose of this bulletin to summarize the present knowledge of clover anthracnose produced by *Colletotrichum trifolii.* The similar disease produced by *Gloeosporium caulivorum* is to be considered in a later paper.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

In 1905 the botany department of the Tennessee Agricultural Experiment Station (*4*) undertook a survey of the State to determine the cause of repeated failures of red clover, which had threatened the success of this important crop throughout many of the best farming districts of Tennessee. In the course of the investigation several diseases were observed producing more or less injury to clover, but their combined damage was found to be of minor importance when compared with the losses caused by a new fungus, *Colletotrichum trifolii*, which was described by Bain and Essary in 1906 (*5*, *6*).

At about the time it was discovered in Tennessee, J. M. Westgate found the same disease causing serious damage on alfalfa in Virginia and sent specimens to Mrs. F. W. Patterson of the Department of Agriculture. Mrs. Patterson later sent her notes and material to Professor Bain, who identified the causal organism of the alfalfa disease as identical with that found in Tennessee on clover (*5*).

In 1905 Sheldon (*20*) reported an undetermined anthracnose fungus found on red clover in West Virginia; the following year he identified *C. trifolii* (*21*).

In 1906 (*5*) the disease was recorded as occurring in Tennessee, Virginia, West Virginia, Kentucky, Arkansas, and Ohio. The following year it was found in Delaware (*9*) and soon afterwards was reported from neighboring States. At present there are records of its occurrence in practically every State in the eastern and midwestern clover belt, with the exception of the New England States, as well as in southern Canada.

Except for a case on alfalfa in South Africa (*1*), the disease has not been reported outside of North America.

HOST PLANTS

Colletotrichum trifolii is regarded as a fungus which attacks principally red clover (*Trifolium pratense*). It is common, however, on alfalfa (*Medicago sativa*) in nearly every section where it occurs on clover. It is also found on crimson clover (*T. incarnatum*), subterranean clover (*T. subterraneum*), bur clover (*Medicago hispida*), and sweet clover (*Melilotus alba*). Recent inoculations in the greenhouse have shown that all of these are readily infected with this organism. No attempt has been made to determine the complete host range of the parasite, although the facility with which it attacks the above-named hosts in three different genera would indicate that it has a much wider range of hosts than is ordinarily assumed. On the other hand, it is by no means an omnivorous parasite even within the Leguminosae. It has not been observed on white clover, and artificial greenhouse inoculations failed to produce the disease on beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), Japan clover (*Lespedeza striata*), Korean lespedeza (*L. stipulacea*), or white lupine (*Lupinus albus*). Alsike clover (*T. hybridum*) is practically immune (*6*).

The inclusion of alfalfa and sweet clover as common hosts for *Colletotrichum trifolii* is an important distinction between this fungus and *Gloeosporium caulivorum;* the latter has so far not been reported on those plants except where based on Saccardo's (*19, p. 1201*) obvious error in listing *Medicago sativa* as the host rather than *Trifolium pratense* which was given in Kirchner's (*11*) original description.

SYMPTOMS

Colletotrichum trifolii occurs on any green part of the plant and on the upper portion of the taproot. It may attack plants at any time in their development from the seedling stage to maturity.

On the leaves the disease occurs as dark-brown or blackened lesions of irregular shape and varying in size from a minute spot to a general infection covering the entire leaf blade. As a rule these lesions are more common near the margin and are frequently more or less angular in shape, conforming to the venation of the leaf. As they commonly occur in the field, there is nothing macroscopically distinctive in these lesions which will serve as a positive identification when found with some of the other leaf spots of clover. In the original description (*5*) it was stated that the disease was rarely found on the leaves, and the statement has since been repeated by several writers. This observation may have been due to confusing the Colletotrichum lesions with some other leaf spots. However, fields are frequently found in which there is a relatively large proportion of leaf infection. Plate 1 shows the disease on leaves of red clover and sweet clover as produced by artificial inoculation with a pure culture of *C. trifolii.* The lesions occurring on sweet-clover leaves are much more sharply defined than in the case of red clover and are a lighter brown in the center, with a distinct reddish brown or black border, especially in the younger dark-green leaves such as are shown on the right in Plate 1, A. This greater color differentiation, as compared with red clover, applies also to a somewhat less extent to the susceptible species of Medicago.

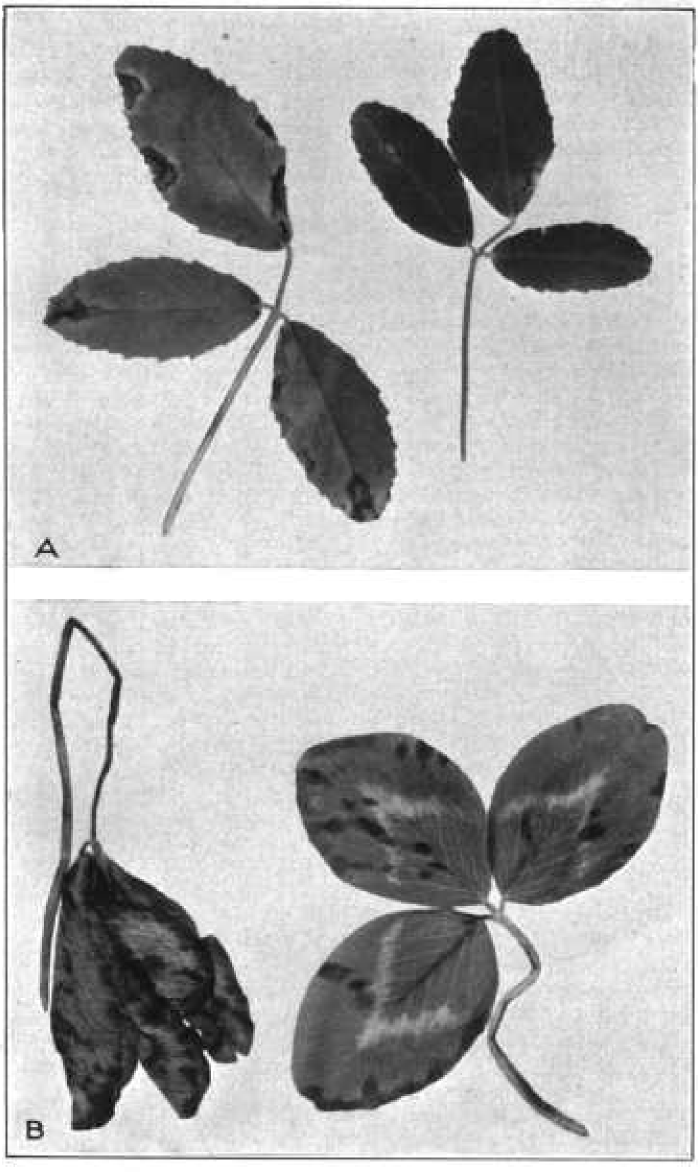
One of the most frequent points of attack is the short stalk of the leaflet. There the lesion may be limited to a single stalk, or it may extend out through the leaf blade or back to the petiole, from where it is likely to pass to the other leaflets. As a result of such infection the leafstalks become black and shriveled, causing the leaflet to droop. Frequently a single leaflet wilts while the rest of the leaf appears perfectly healthy, but as a rule all three are ultimatey involved. (Pl. 2, A.) The infected stalk soon becomes brittle, so that the leaflet is readily broken off by wind or in the process of haymaking.

On stem and petioles the disease first appears as small water-soaked spots. These as a rule rapidly elongate to form long depressed lesions which soon become dark brown or black. On the larger petioles and stems they may become distinctly zoned, with a light-brown or gray center and a dark-brown or black border. In the center of such lesions the fruiting of the fungus is ordinarily clearly evident, its clusters of dark setae and masses of pink spores being readily seen under a hand lens or even with the naked eye. The centers of older lesions are usually deeply sunken and cracked frequently into the pith. The disease is most common on the young succulent parts of stems or petioles, although under favorable conditions it may attack even mature tissue. As a rule, especially on rapidly growing plants, there is a characteristic curling of the stem or petiole about the point of infection, producing a distinct hook and causing a drooping of the parts above even before they start to wilt. This is illustrated in Plates 1 and 2. The affected tissue usually dries out rapidly and becomes so brittle that leaves are readily broken off. When the disease is well developed the parts above the point of infection dry out and turn brown.

In most cases the lesions on the stems and petioles of alfalfa are smaller and not as deeply sunken in the center as on clover. The parts above, nevertheless, usually wilt, and the leaves become pale and bleached. (Pl. 3.) As in clover, the most serious damage on alfalfa is caused by infection of the crown (*22*).

Infection may also occur on the crown or upper portion of the taproot. When it affects the crown the fungus produces a general rotting which in most cases results in the death of the entire plant. The crown rot is closely correlated with taproot infection and may result from growth of the fungus down from the stems and petioles or upward from the root. On the upper part of the taproot dark lesions are produced, which gradually encircle the root and ultimately cause the entire plant to wilt and die. The disease on the crown or taproot may for a time develop on only one side of the plant, causing that side to wilt, while the rest may develop in an apparently normal manner. (Pl. 4.) These infections, as a rule soon spread and involve the entire plant or at least so weaken it that it is unable to withstand the winter or other unfavorable climatic conditions; or they may open the way for invasions by weak parasites. As in the case of petiole and stem lesions, the diseased tissue becomes extremely brittle, and the plant is readily broken off at the soil level.

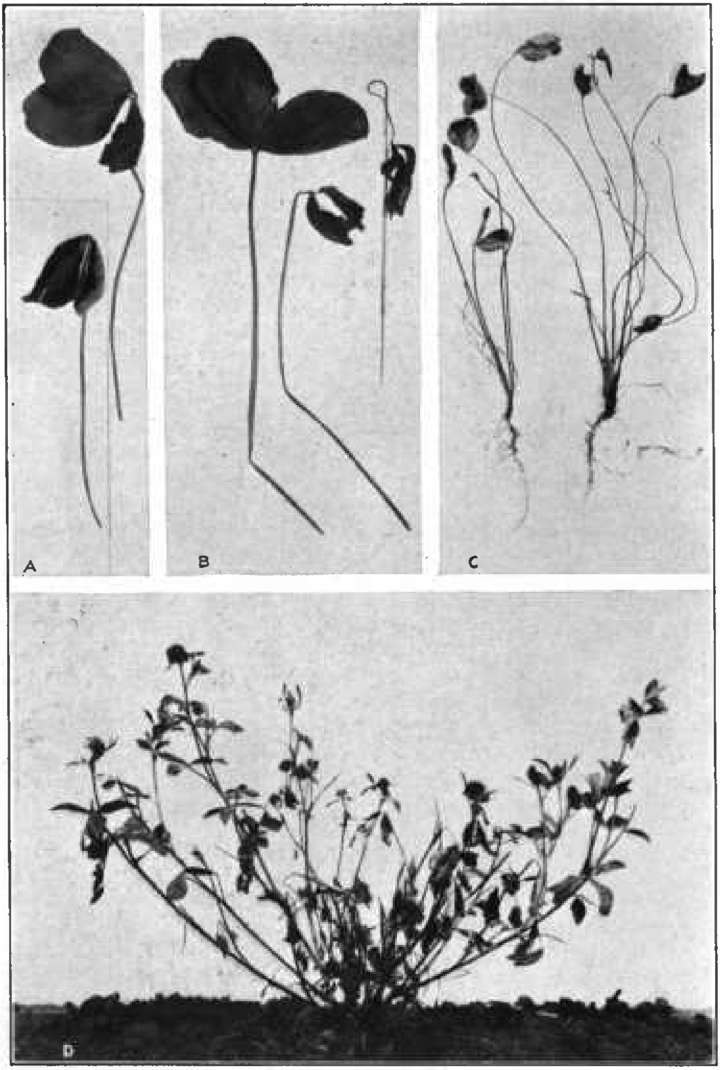
In fields severely affected with anthracnose the first and most conspicuous symptom is that of drooping leaflets or flower heads. The



ANTHRACNOSE ON LEAVES AND PETIOLES

A, Lesions produced by artificial inoculation on leaves and petioles of *Melilotus alba*. The leaf on the left was mature when inoculated and when photographed was beginning to turn yellow because of lesions on the petiole. The younger leaf on the right was a darker green and shows the distinct black border which was especially prominent around lesions on the newer leaves. Note also the concentric zonation in the larger lesions and the halo around some of the spots produced by a yellowing of the leaf in the vicinity of the lesion

B, Lesions produced on *Trifolium pratense* at the same time and with the same inoculum as in A, showing the decided susceptibility of the leaf blades to infection by this organism. On these lesions note the absence of the distinct borders such as those on the leaves of sweet clover. Severe petiole infection is evident on both leaves. The one at the right shows the characteristic curling of the petiole about the point of infection, while that on the left shows a somewhat more advanced stage and the resulting drooping of the leaf. Note on this petiole the speckled appearance of the lesions produced by the masses of setae of the fungus



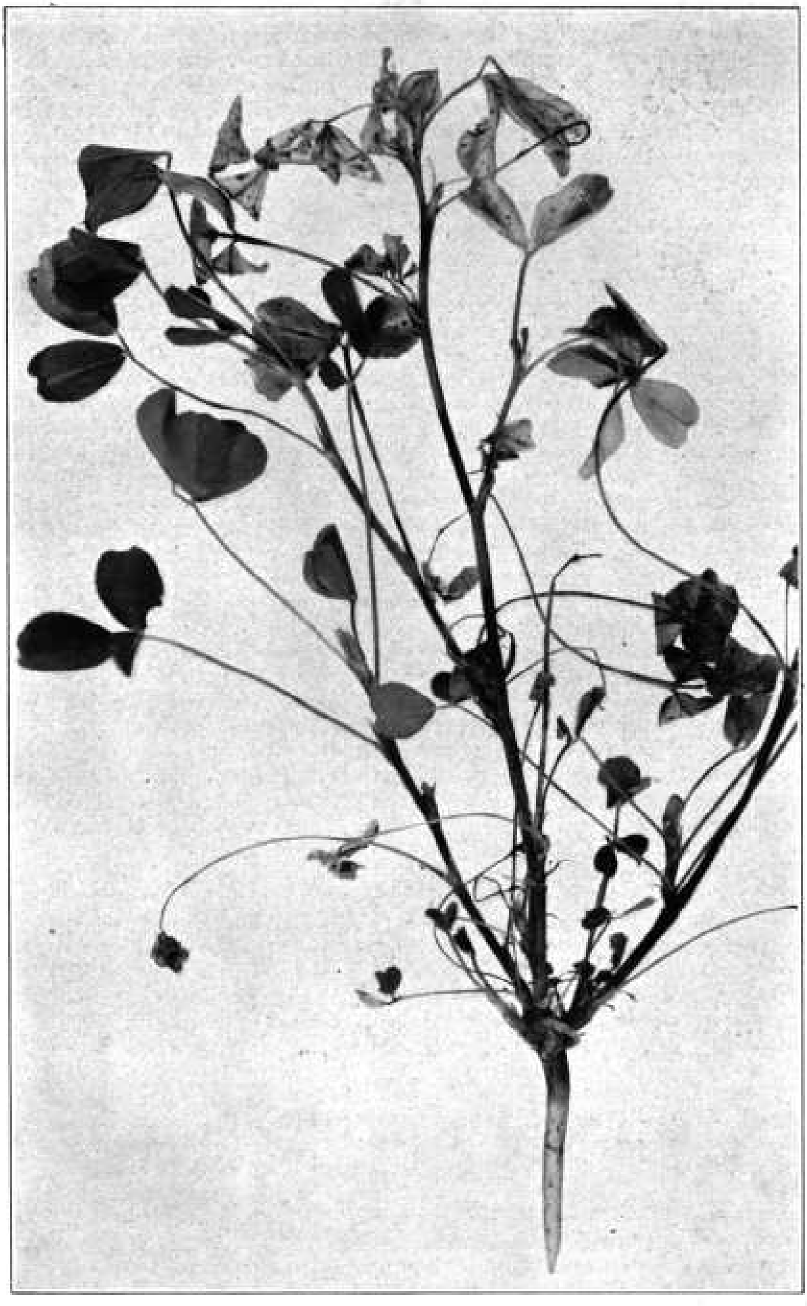
ANTHRACNOSE ON CLOVER

A, Lesions on the stalks of the leaflet. On the upper leaf infection started on the stalk of a single leaflet, causing it to wilt, while the other leaflets remained turgid. The disease, however, had spread into the other two stalks and leaflets. The lower leaf shows simultaneous infection of all the stalks, causing the leaflets to wilt while the petiole remained healthy

B, Petiole infection. The two specimens at the right show the characteristic curling of the petiole at the point of infection, and the wilting of the leaves. The leaf at the left shows an infection which has encircled the petiole but which has as yet caused no serious collapse of the parts above. Such leaves quickly wilt in periods of dry weather

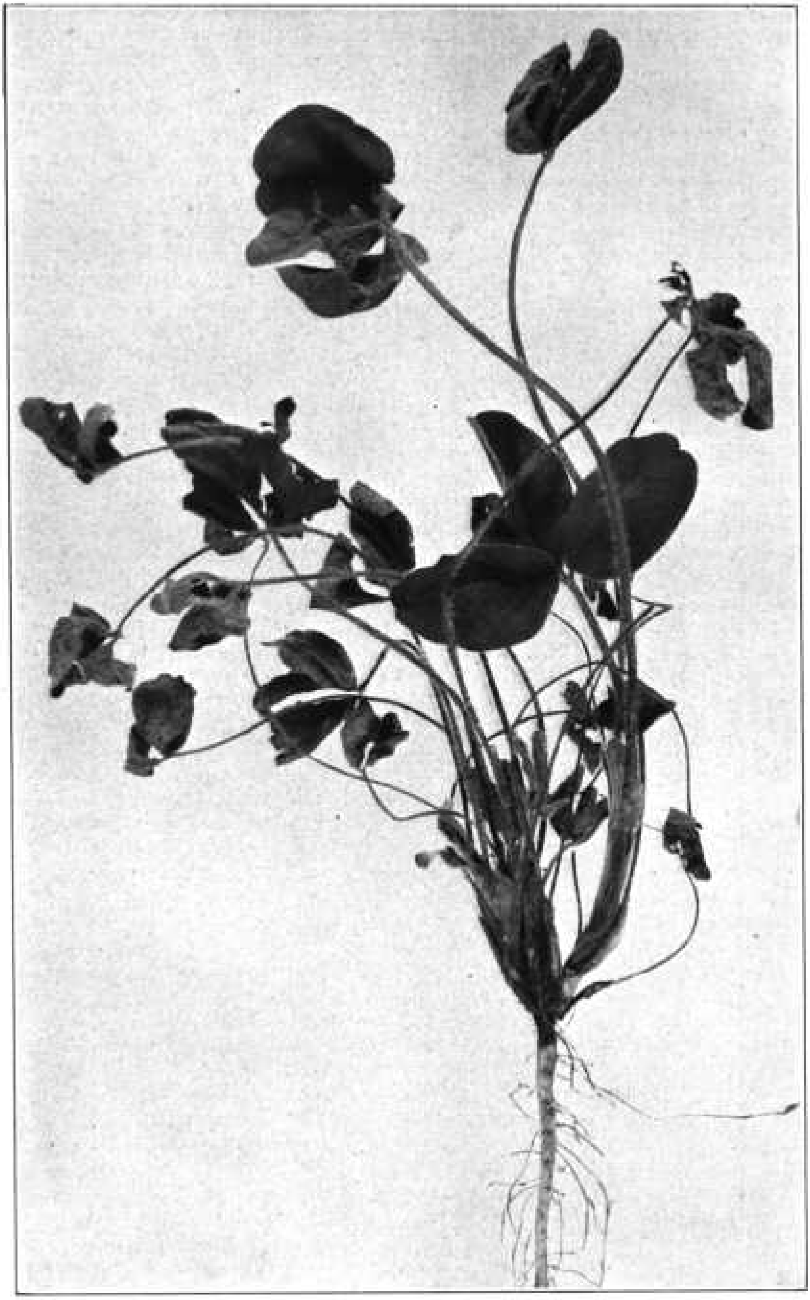
C, Anthracnose on clover seedlings. Typical destruction of the leaves during the summer months by repeated petiole infections is shown. These plants were photographed in August from a spring planting at Arlington Experiment Farm. Note the healthy condition of the taproots and crown with the new healthy leaf coming from the crown of each plant. Even in the absence of root or crown infection such plants are seriously weakened by the repeated pruning of the leaves by anthracnose

D, Anthracnose on clover in its second season, after hay harvest. Note the general infection, especially among the younger stems near the center of the plant. It is this type of infection which frequently destroys a stand of clover after it has produced a satisfactory yield of hay and results in loss of seed crop and greatly reduced value of the stand for green-manure purposes



COLLETOTRICHUM TRIFOLII ON ALFALFA

Lesions on the stem and peticle and resulting wilting of the leaves above the affected parts. The central stem shows three dark lesions with the bleached, wilted leaves above them. The lesion in the center of the second internode of the stem at the right shows black dots, indicating clusters of setae of the fungus. Lesions are also shown on several of the petioles



ANTHRACNOSE OF THE CROWN AND TAPROOT OF RED CLOVER

There is a well-defined dark lesion on the upper part of the taproot and rotting of the crown. Note also the branch at the right, which as yet shows no wilting due to the crown or root infection. This plant, photographed in August, 1925, was from a plot seeded in the spring of that year. At the time the photograph was made more than 50 per cent of the plants in many of the plots showed infection of this type dark shriveled lesions and the resulting curling of the stem or petiole serve readily to distinguish anthracnose from the only other clover injury likely to be confused with this symptom; that is, the feeding of clover weevils on the stem or petiole. These drooping parts soon wilt, turn brown, and as more serious infection kills whole stems or entire plants the field becomes spotted with browned areas. In severe cases fields appear as if injured by fire, which has led to the name "scorch" being used in some localities to designate anthracnose. The drying of the plants, especially where the attack is general on roots or crowns, is readily confused with that produced by lack of moisture, and undoubtedly many of the losses of clover stands attributed to drought are in reality the result of injuries produced by anthracnose. Confusion with sun scald or "drying out" is often due to the fact that in periods of moist weather the disease may develop to a serious degree without causing noticeable wilting of the leaves, as is shown in Plate 2, B. When hot, dry weather follows a period of general infection and development of the disease a large proportion of the leaves may suddenly collapse because of the inability of the injured conducting tissue to maintain a supply of water to take care of the increased transpiration.

The macroscopic symptoms of anthracnose produced by *Colletotrichum trifolii* and *Gloeosporium caulivorum* are so nearly identical that positive identification in the field is generally difficult, if not impossible. Lesions produced by the Gloeosporium are frequently longer and are likely to be associated with long brown streaks extending well down the petiole or stem. This distinction apparently is by no means constant and is therefore unreliable. On older lesions the black tufts of setae of *C. trifolii* are at times readily distinguished by the naked eye and are clearly visible with the aid of a hand lens. The presence of these dark clusters serves as a ready means for identifying the Colletotrichum under field conditions, but their absence does not necessarily serve to identify Gloeosporium, since there are many occasions when the setae produced by *C. trifolii* are not sufficiently prominent to be recognized except with the aid of a microscope. However, on lesions which are well developed and when conditions are favorable for spore production the absence of these tufts offers a fairly reliable means for identifying *G. caulivorum*. Positive identification must usually depend on microscopic examination. (Fig. 1.)

INJURY PRODUCED

The fungus penetrates directly through the epidermis, in the manner common to this type of fungus, and by rapid intercellular and intracellular advance soon breaks down the epidermal and outer cortical cells near the point of penetration. It soon reaches the phloem and region of the cambium, where it immediately causes collapse of the tissue, which results in the sunken appearance of the lesion. Its longitudinal and lateral extension is primarily through the outer cortex. The mycelium penetrates into the pith and lastly affects the xylem. Plate 2, B, shows a type of lesion where the mycelium has destroyed the entire parenchymous tissue but where the xylem is still capable of functioning sufficiently to maintain the turgidity of the leaf. The rapid progress of the fungus through the cortex and the resulting collapse of the cells on one side produce the characteristic curling of the petiole or stem about the point of infection. This is most noticeable when infection is on rapidly growing tissue, for in such cases growth is stopped on one side of the stem or petiole but continues on the uninjured side, thereby forcing a distinct curl. The drying of the injured tissue often results in deep longitudinal cracks through the lesion, frequently extending well into the pith.

As ordinarily found in the field, infection of the leaf blades is of relatively minor importance. The disease on stalks of the leaflet or on petioles is of much more serious consequence, especially on seedlings. In a thick stand where conditions are most favorable for infection, petioles and leaflets may be cut off almost as rapidly as they are produced. (Pl. 2, C.) This constant pruning of leaves greatly reduces the vigor of the plant, which, even if it survives the repeated attacks throughout the summer, is in a much less vigorous condition for overwintering. If, however, the attack is limited to leaves and petioles, the plant may entirely recover before winter and there may be little damage, for the loss of foliage as a result of disease is a setback no greater than is the common practice of pasturing the crop in early fall. The most serious losses during the seedling year are those due to crown or root rot, which when severe leaves little chance for recovery. General petiole infection, such as is shown in Plate 2, C, commonly results in a large percentage of diseased crowns, for this dead material forms a mat over the young plants, and the old lesions produce sufficient inoculum to infect the new leaves as they push through. When young clover is so hampered, the disease readily works back through the petioles to the crown, where a general rotting quickly destroys the plant. This injury, together with that of the taproot, results in a severe thinning of the stand during the summer months and is responsible for much of the dying in early fall. It is also likely that some of the so-called "winter injury" is indirectly due to anthracnose on the taproot or crown which, though not developed sufficiently to cause death, may be so weakening that during the period from late fall to early spring the plant is more likely to be affected by climatic conditions and parasites. This reduction in vigor of seedlings during the critical period of the

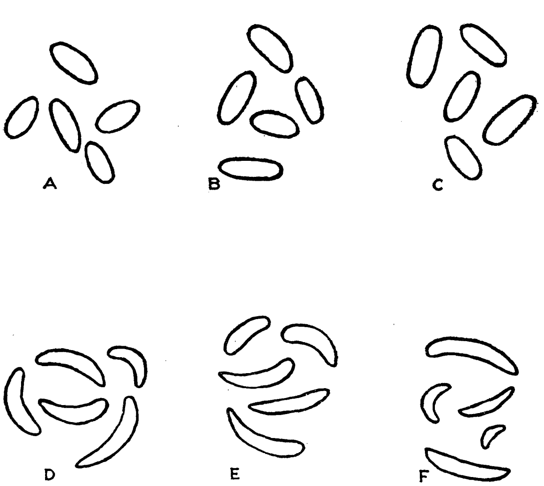


FIG. 1.—Spores of *Colletotrichum trifolii* (A, B, C) and *Gloeosporium caulivorum* (D, E, F), showing differences in size and shape, regardless of host or different environment. A, from alfalfa, Appomattox County, Va.; B, from red clover, Lexington, Ky.; C, from red clover, Arlington Experiment Farm, Rosslyn, Va.; D, from crimson clover, Montross, Va.; E, from red clover, Aberystwyth, Wales; F, from red clover, Wooster, Ohio. (Camera-lucida sketch, × 575)

year also makes them more likely to be crowded out by neighboring plants or weeds.

During the second year the disease by means of stem and leaf infection may greatly reduce the hay harvest. In most seasons, however, this injury is of little consequence. The heavy loss comes after the first cutting, when the plants are producing a new growth. (Pl. 2, D.) At such times stem infection may be an important factor, but by far the most serious loss is due to crown or taproot infection, after which there is no recovery.

Since the production of hay is only one of the functions of a clover crop, a measure of the reduction in hay yield represents often only a small part of the total loss. Under certain conditions a fairly good hay crop may be obtained before the disease causes any severe thinning of the stand. Later development of the disease, however, may destroy the plants in such numbers that the second crop is practically worthless. Such a condition not only prevents harvest of a seed crop but materially reduces one of the chief values of the crop in a rotation system, namely, that of soil improvement. Destruction of the stand during the seedling year may be avoided to some extent by fall planting, but if during the following summer the plants are killed soon after the first cutting the crop is suitable only for a short rotation system, for which purpose such legumes as crimson clover are better adapted.

THE CAUSAL ORGANISM

TAXONOMY

The taxonomic position originally assigned the fungus by Bain and Essary (*5*) has not been questioned by anyone working with it. Before reporting their investigations they compared the new Colletotrichum with specimens of the clover disease described by Kirchner (*11*) as due to *Gloeosporium caulivorum*. In spite of this, some pathologists regard these fungi as identical or as strains (*8*, *10*, *23*). The symptoms produced by the two diseases are very similar, and since the distinction between Gloeosporium and Colletotrichum is based on the somewhat unstable character of setae production, it has been assumed at times that the presence of setae was a response to different environment and that the fungus was in reality the same as that described by Kirchner. However, the original description of *Colletotrichum trifolii* was based also on differences in size and shape of conidia.

In the investigations here reported it was found that *C. trifolii* consistently produced setae when sporulating under all the variations of temperature or other environment, both on different hosts and on artificial media. Similar observations of *G. caulivorum* did not reveal setae in a single case. The spores of *G. caulivorum* show considerable variation as to size and shape, while those of *C. trifolii* are relatively constant in both these characters. Furthermore, there is a distinct difference in spore production in these two species, the Colletotrichum spores being produced one at a time from the tips of the conidiospores, whereas in the case of the Gloeosporium several spores are frequently produced simultaneously from a conidiosphore. The host range of these two fungi is also different. *C. trifolii* readily attacks alfalfa, sweet clover, and other related legumes, but so far *G. caulivorum* has not been found in the field, nor was it produced by artificial inoculation on any host other than species of Trifolium, particularly red clover and crimson clover. It is therefore evident that these two fungi are well-separated species.

There are, however, other species of Colletotrichum on clover which more closely resemble *C. trifolii* and are more readily confused with it than is *G. caulivorum*. O'Gara (*16*) reported a new species of Colletotrichum on clover in Utah which was regarded by Professor Bain as a distinct species. *C. cereale* has also been reported on clover. Specimens of an anthracnose of bur clover were received from O. C. Boyd, who found the disease at Metcalf, Ga., in March, 1925. In a letter accompanying the specimens he reports that "25 per cent of the plants were affected," but that the disease occurred "only on this clover in a field with alfalfa, vetch, melilotus, and about a dozen clovers." The disease was found to be produced by a Colletotrichum having spores noticeably longer than those of *C. trifolii*. Differences were also noted in growth in culture media. Pathogenicity tests by artificial inoculations showed that while *C. trifolii* attacked bur clover as readily as it did red clover, the other was unable to infect red clover, although producing very severe injury on bur clover.

It appears probable that there are several fungi similar to *C. trifolii* occurring within its host range, with differences no doubt sufficient to justify specific rating. During the course of the present investigations of the Colletotrichum commonly found on red clover no evidence has been noted of the existence of different strains, unless those above mentioned as distinct species are regarded as biological forms of *C. trifolii*.

ISOLATIONS

The fungus is readily isolated on most of the common agars. In this work isolations were made from new lesions on red clover according to the usual procedure of diluting spore suspensions and making single-spore transfers to potato-dextrose agar. Cultures were prepared from material obtained in several localities, but since their behavior was similar most of them were discarded and that isolated from specimens collected at Arlington Experiment Farm, Rosslyn, Va., was used for practically all inoculations and culture studies.

SPORE GERMINATION

Conidia germinate readily when taken from either fresh or old lesions or from artificial media. Germination tests were made by placing drops of the spore suspension on glass slides kept in Petri dishes containing moist filter paper to maintain a suitable humidity. The spore suspensions were made in distilled water, in decoctions from clover, alfalfa, or melilotus stems, in water containing sap squeezed from fresh stems of clover or melilotus, and in drops of water containing fragments of clover petioles or leaves. The addition of any material from these host plants, fresh or cooked, increased the rapidity and percentage of germination as compared with that obtained in distilled water. There were occasional exceptions to this, for when fragments of a resistant clover were placed in the water suspension there was frequently a decrease in spore germination, as previously pointed out by Bain (*3*). The rate and percentage of germination are affected by temperature and moisture, as will be shown later under the discussion of the influence of these environmental factors on the development of the disease. The conidia are apparently able to germinate equally as well in daylight as in total darkness.

CULTURAL CHARACTERS

At ordinary room temperature, on plates of potato-dextrose agar, conidia germinate within four to eight hours, sending out one to three germ tubes. In less than 48 hours the colonies become macroscopic. The germ tubes and younger hyphae are hyaline, but the mycelium in the center of the colonies is more dense and produces thick clusters of hyphae which soon become faintly colored. These masses of mycelium are macroscopic in three to four days and appear as olivaceous or dark-green stromata. As the colony grows these stromata increase in number, occurring everywhere except along the outer border of the culture. They become larger, darker, and more numerous with age, and together with the tufts of dark setae, soon cover the center of the colony with a thick black stroma on the surface of the agar.

In four days conidia are produced at the center and continue to appear in abundance on all but the outer portion of the culture. The older parts are usually covered with pink masses of spores, and as the colony develops, tufts of white aerial mycelium appear, but seldom in sufficient quantities to mask the dark stroma or pink masses of conidia.

Growth of cultures on slants of potato-dextrose agar appears very similar to that on plates of the same medium. The mycelium is limited to the upper layer of agar and produces a surface stroma as it does on the plate cultures. The pink spore masses are even more conspicuous in tubes, whereas the aerial mycelium is usually more limited.

The fungus grows readily on numerous other artificial media, including cornmeal or oatmeal agar, synthetic agars, beef-broth agar, and various cooked or fresh plant tissues. Differences on the various agars are simply those of rate of growth and spore production, variations not strikingly distinctive from the usual behavior of other species of Colletotrichum in culture. Sporulation is especially abundant on cooked stems of clover, alfalfa, or sweet clover. On these media the aerial mycelium is reduced to a minimum, and the stems become coated with thick masses of pink spores and an abundance of setae. This type of medium, used in the early work in Tennessee, is the most suitable for the production of large quantities of spores for artificial inoculations.

RELATION OF TEMPERATURE TO GROWTH ON MEDIA

To test the effect of temperature on the growth of *Colletotrichum trifolii* on potato-dextrose agar, a series of Petri dishes, each containing 15 cubic centimeters of the medium, was inoculated with fresh spores and placed in an Altmann incubator at constant temperatures. The first tests were conducted in compartments maintained at temperatures with 4-degree intervals between 4° and 32° C. No growth occurred at 4° or 8°, but the fungus was able to grow at all the other temperatures in the series (*13*). Plate 5, B, shows a typical group of cultures 8 days old selected from one of the temperature series. It will be seen in this halftone that growth was relatively slow at 12° but gradually increased at the higher temperatures up to an optimum at 28°. At 32° there was less growth than at 28°, although this temperature was still favorable for development of both mycelium and spores. At 12° and 16° sporulation did not occur.

Similar tests were made, pieces of mycelium being used in place of the conidia for the inoculum. A corresponding response to temperature resulted, except that in this case the colonies started somewhat earlier and maintained this lead throughout the experiment. Sporulation was practically the same whether the transfer was made from mycelium or from spores of the same colony.

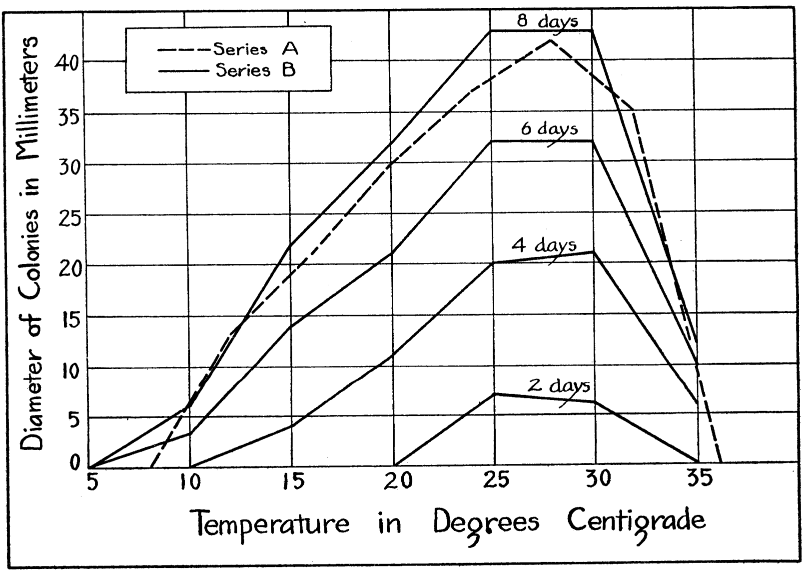


FIG. 2.—Effect of temperature on growth of *Colletotrichum trifolii* on potato-dextrose agar

The influence of temperature was also determined on sweet-clover agar, malt agar, and cooked sweet-clover stems, as compared with the standard potato-dextrose agar. In each case the optimum and extreme temperature limits were practically the same, differences being merely those of variations in growth due to differences in the the available food, regardless of temperature.

In later work growth was compared at temperatures maintained at 5-degree intervals, from 5° to 35° C. This change was made to correspond with the temperatures maintained in a series of moist chambers where the effect of air temperature on infection by this organism was being studied. Figure 2 shows the rate of growth based on measurements of greatest frequency of cultures on potato-dextrose agar. In it are combined the growth measurements for the temperature series at the earlier 4-degree and the later 5-degree intervals.

EFFECT OF LIGHT

Bain (*2*) found that the spores of the fungus were extremely sensitive to direct sunlight. Although his results applied primarily to excessive heat from sunlight striking the spores, it was thought advisable to test the possibility of the influence of light on spore germination and rate of growth. To do this a series of Petri-dish cultures was inoculated with fresh conidia and placed in moist chambers in the greenhouse. These were maintained at 15°, 20°, 25°, and 30° C., and at the same time a similar set of cultures was placed in the Altmann incubator at these same temperatures. The set in the inoculation chambers was not exposed to direct sunlight but to daylight passing through two layers of ordinary greenhouse glass. Measurements for the two series showed that cultures in the light grew at practically the same rate as those grown in total darkness. Similar tests on the effect of light on spore germination showed that the response to temperature was practically identical in the light and dark chambers.

This series also furnished a check on the possibility of an inhibitory effect due to drying of the media at the higher temperatures in the incubator. The air in the inoculation chambers was maintained at the point of saturation, and therefore the culture media did not lose moisture. The results showed that evaporation from the media in the Altmann chambers, even at the higher temperatures, was not sufficient to influence noticeably the growth of the fungus in the short periods used in these experiments. Spores in suspension in drops of water or plant decoctions when allowed to dry down did not germinate even in an atmosphere with a relative humidity of 90 per cent.

EFFECT OF ACIDITY

The fungus is able to grow on culture medium having considerable variation in acidity. Plate 5, A, represents a series growing on potato-dextrose agar in which the P_H ranged from 3 to 9.5. This series was prepared according to the usual potato-dextrose agar formula, provision being made for mixing in the necessary amount of HCl or NaOH at the time the plates were poured. Part of the media was retained and tested immediately by the colorometric method, to determine the degree of acidity just before the agar hardened. There were only negligible differences in rate of growth between 3.7 and 6.6. There was, however, a reduction in sporulation on the 3.7 agar, and on the more acid plates spores were not produced.

PATHOGENICITY

In the early work on this fungus in Tennessee its pathogenicity on clover was demonstrated by laboratory and field inoculations. In the present investigation, inoculations of spores sprayed on plants by means of the usual atomizer method were compared with those made by smearing or spraying the inoculum over scalpel cuts or needle punctures in leaves, petioles, and stems. These experiments showed conclusively that this fungus needs no injury for penetration nor weakened host tissue for its further development. Although more likely to attack the new succulent growth, it may develop on the old parts whenever climatic conditions are favorable. When penetration is effected, the organism rapidly breaks down the tissue of the host, and within four days distinct macroscopic lesions are produced. Its pathogenicity has been demonstrated on *Trifolium pratense*, *T. pratense perenne*, *T. incarnatum*, *Medicago sativa*, *M. hispida*, *Melilotus alba*, and to some extent on *T. hybridum*. Inoculations of *Phaseolus vulgaris*, *Pisum sativum*, *Lupinus albus*, *Lespedeza stipulacea*, and *L. striata* failed to produce any disease symptoms.

THE FUNGUS IN RELATION TO ANTHRACNOSE

SEASONAL DEVELOPMENT OF HOST AND PARASITE

Red clover as ordinarily used in American agriculture is a biennial. It is planted in the spring or late summer and the following year yields two crops; the first harvest is used for hay, and the second growth is usually allowed to produce seed or is plowed under for soil improvement. Therefore, where spring seeding is practiced the crop passes through two summers. Clover makes its most vigorous growth during the cooler seasons of spring and fall and is greatly handicapped by periods of hot dry weather. Certain of the imported strains of red clover, especially Italian, are much more noticeably checked by the severe conditions of midsummer. *Colletotrichum trifolii*, on the other hand, is favored by higher temperatures and is practically harmless in cool weather when clover thrives best. The periods during the summer months when the temperature is most favorable for the parasite and least favorable for the host are therefore the times when the disease causes greatest losses. In the seedling year such a period frequently follows the removal of the nurse crop, and the sudden added exposure to sun and wind tends to weaken the young plants, making them more likely to succumb to an attack of anthracnose. During the second season the plants are usually exposed to trying weather conditions soon after the first cutting. Wherever anthracnose is widespread under such conditions a large part of the stand is frequently lost, and if its ravages continue throughout the summer there is little likelihood of a third crop being produced for turning under in the fall or following spring. If the plants pass through the first summer without infection of the crown or root they are usually able to recover entirely from the disease during the cool fall months and have adequate growth to enable them to overwinter safely. If, however, the disease has reached the crown or taproot, the plant has little chance of complete recovery and at any time during the fall or winter may die, owing to the gradual development of this fungus or to secondary parasitic invasions.

SPORE PRODUCTION

Under favorable conditions of temperature and moisture the complete cycle from infection to spore production may occur within four days. Conidia are produced abundantly even on small lesions, and in periods of high humidity they are produced in enormous quantities on newly developed lesions as well as on dead plants.

Thus far the fungus has been found to produce only the conidial type of spore. In 1911 Bain (*2*) reported the discovery of perithecia which he thought were the perfect stage of *Colletotrichum trifolii*, but later work failed to corroborate this observation.[3]

VIABILITY AND LONGEVITY OF MYCELIUM AND CONIDIA

The fungus retains its viability for a long period in dried clover tissue. Herbarium specimens several months old readily produce conidia when put in a moist chamber. Fragments of diseased plant tissue which are occasionally carried with seed are therefore possible sources of contamination. Bain showed that conidia were very sensitive to direct sunlight and high temperatures. On the other hand, stem lesions collected in the field in extremely hot weather contained viable spores in large quantities, so this sensitivity to high temperatures is by no means sufficient to entirely check the disease under field conditions. The manner of overwintering has so far not been determined, but all available evidence indicates that the fungus lives over winter as conidia or as mycelium in living or dead host tissue. Attempts to overwinter conidia and mycelium of this fungus on clover stems in flasks at Madison, Wis., were unsuccessful, whereas *Gloeosporium caulivorum* under similar conditions readily survived. Infection experiments in which were used diseased clover plants overwintered at the Arlington Experiment Farm showed that the fungus survived in such material in that latitude. Examination of these diseased plants failed to reveal any but the common spore form.

DISSEMINATION OF CONIDIA

Probably the most serious factor in local spore distribution is spattering rain. At times of high humidity the fungus produces multitudes of spores which if undisturbed tend to cling together even when dry. These spore masses are rapidly broken by falling rain, and the spores are washed to healthy parts of the plant or to the ground, whence they may be carried to other plants by spattering water. A light shower following a period of active spore production appears to be responsible for a large part of the spread of the disease from isolated infection centers. Under certain conditions it is possible that the conidia are carried directly by wind, as are various rusts or mildew spores. This method, however, apparently is of minor importance in the case of clover anthracnose. The wind undoubtedly many times is an important agent in distributing the disease in a different manner; that is, by carrying dried infected clover stems or leaves which upon settling in a moist situation serve as centers for further dissemination. Insects and various animals undoubtedly at times share in the distribution of conidia.

METHOD OF INFECTION AND PERIOD OF INCUBATION

Infection usually takes place directly through the uninjured epidermis. Diseased spots are often found originating at the edge of insect punctures or other wounds on leaves or stems. Except in tap-

[3] Correspondence from S. H. Essary, Apr. 23, 1926.

root and crown infection, such cases are of minor importance in the development of the disease. Experiments in which leaves and petioles of a number of plants were pricked with a needle before spraying them with a suspension of spores showed that such injuries did not cause any appreciable increase in infection as compared with similar uninjured plants inoculated in the same manner. The percentage of crown and taproot infection, however, was greatly increased by punctures or cuts previous to inoculation. Incisions made by various worms or insects feeding about the crowns of clover plants undoubtedly open the way for penetration of the fungus into these parts and greatly aid serious infections of the taproot, particularly during the second season.

Under favorable conditions the incubation period is from three to five days. It is, however, readily modified by slight changes in the temperature at which the plants are kept after inoculation. On leaf blades the disease becomes evident more quickly than on stems or petioles. Under field conditions, lesions are produced in abundance at any time during the summer months within three to seven days after periods of warm weather with sufficient dew and showers to favor infection.

SOURCE OF NATURAL INFECTION

There is considerable evidence to indicate that clover anthracnose is carried with seed from infested fields. To what extent this is a factor in the distribution of *Colletotrichum trifolii* has not been experimentally determined. A few attempts have been made to study this phase of the problem by gathering seed heads from badly diseased plants. Owing to the fact that the stem just beneath the flower head is extremely susceptible, any infection near the flower usually affects the stem beneath, and its development quickly cuts off the water and food supply for the blossoms, consequently seeds are seldom produced. It is, therefore, unlikely that the disease is actually transmitted within the seed to any such serious extent as is often assumed. Certainly in the case of clover it is not a factor of nearly so great importance as in the case of anthracnose on other legumes such as beans. On the other hand, it undoubtedly is frequently carried with the seed, primarily by means of fragments of diseased stems or petioles which are broken off in the process of seed harvesting and passed through the cleaning machinery.

Experiments at the Arlington Experiment Farm in the spring of 1924 indicated that soil and plant refuse from a previously diseased clover crop carried sufficient inoculum to contaminate thoroughly a new seeding. Diseased leaves are easily broken off when dry and may readily be carried by wind to neighboring clover fields. Such leaves, if they settle in moist places among a thick stand of clover, will produce an abundance of inoculum to infect near-by plants. These leaves are often seen blowing from a badly infested field, especially during hay harvest, and they undoubtedly play an important part in distributing the disease throughout a clover field or in carrying it to new areas.

ENVIRONMENTAL FACTORS INFLUENCING OCCURRENCE AND PROGRESS OF THE DISEASE

TEMPERATURE

Colletotrichum trifolii has been reported chiefly from the southern section of the clover belt. It has frequently been found in more northern sections, including Wisconsin, Michigan, and Canada, but in these localities it seldom becomes a serious pest. Thus far it has never been observed in Europe, although the anthracnose produced by *Gloeosporium caulivorum* is there considered one of the most injurious diseases of clover. In States where common it becomes a menace to the crop only during the summer months, especially in hot humid weather. The geographical distribution and the seasonal appearance of the disease indicate that temperature may be one of the deciding factors in its development.

To determine the relationship of temperature to infection and development of the disease, four moist chambers were constructed in which the air was maintained constantly at definite temperatures by means of electric thermostatic-control equipment, the humidity being kept at the point of saturation. These chambers, being largely of glass, provided sufficient light to enable plants to thrive in them for weeks at a time, although direct sunlight was shaded from the plants. By this means the light, moisture, and other factors other than air temperature were made approximately the same for each of the four chambers.

For most of the work these chambers were maintained at 15°, 20°, 25°, and 30° C., the intervals of 5° being sufficiently wide to make any slight variations in temperatures a negligible factor in comparing results. Pots containing five seedling red-clover plants each were used for the tests, and these were sprayed with suspensions of spores from fresh potato-dextrose agar cultures. The pots were then placed in the different chambers and as a rule kept there until the disease developed. Table 1 shows the result of a typical series with red clover, indicating a decided influence of air temperature on infection. Figure 3 shows the relationship of temperature to spore germination at intervals of six hours. It will be seen that spores germinated most rapidly at 25° C., as indicated by the count made at the end of the first six-hour period. After 12 and 18 hours the counts did not show as much variation, and total germination was approximately the same at 25° and 30° C. Figure 4 shows the influence of temperature on spore germination, on growth of mycelium on agar plates, and on leaf and petiole infection of clover seedlings. The general trend of the curves is similar. As shown in the graph, the response to temperature, indicated by the number of lesions produced, was much more striking than that of either spore germination or mycelial growth. This was especially so in the case of infection at 25° and 30° C. Other series with a different range of temperatures indicate that the optimum for growth on agar is somewhat above 25°, approximately 28°, as shown in Figure 2. Infection experiments at these temperatures also indicated that the optimum temperature for infection was the same as that for optimum growth on the agar plates. Plate 6 shows the rapid progress of anthracnose at 24° contrasted with the much-delayed development at 18°.

TABLE 1.—*Influence of temperature on infection of red-clover seedlings by Colletotrichum trifolii*

The number of distinct lesions shown represents those evident on five plants, the plants having an average of 25 leaves]

Period of incubation	Number of lesions							
	At 15° C.		At 20° C.		At 25° C.		At 30° C.	
	On leaves	On petioles	On leaves	On petioles	On leaves	On petioles	On leaves	On petioles
4 days	0	0	0	0	25	0	0	0
5 days	0	0	0	0	325	35	0	0
6 days	0	0	15	3	700	250	10	2
7 days	0	0	80	5	(1)	(1)	10	6
8 days	0	0	125	5	(1)	(1)	10	8

[1] The lesions had become so numerous and had coalesced to such an extent that counts were not attempted.

Under field conditions this response to temperature is clearly evident. The disease does not ordinarily develop to a serious extent in the latitude of Washington, D. C., until the latter part of May or early June. Throughout the summer it is most conspicuous following hot humid weather. On the other hand, the periods of excessive heat are not always those most favorable for the disease, for at such times the humidity is frequently too low. A temperature of 25° to 28° C. during cloudy weather or at times of frequent showers and heavy dews invariably results in an enormous increase in infection. This requirement of a high temperature combined with abundant moisture is probably the most important factor in limiting the geographical distribution (*18*) of this fungus as an important clover para-

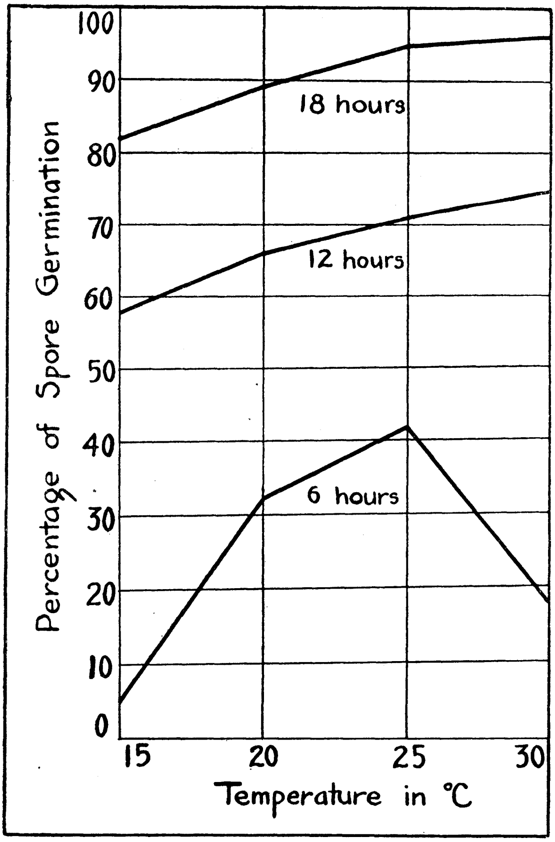
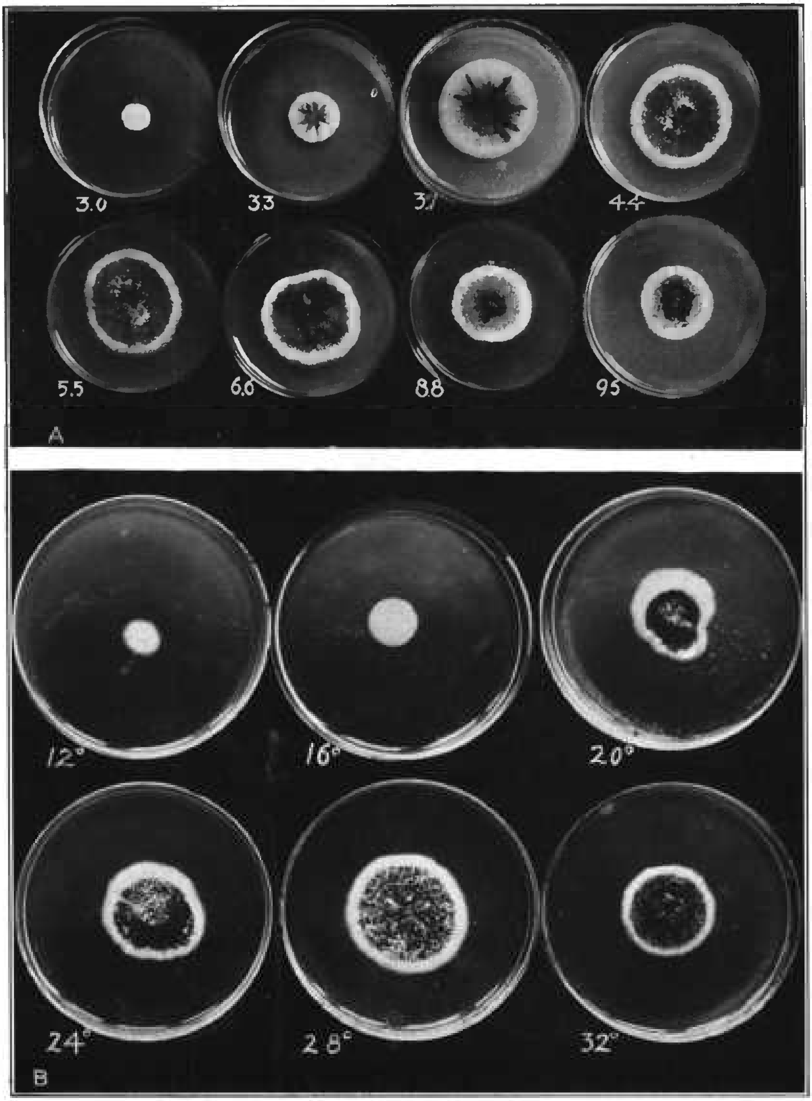


FIG. 3.—Effect of temperature on germination of spores of *Colletotrichum trifolii*



GROWTH OF COLLETOTRICHUM TRIFOLII AS AFFECTED BY ACIDITY AND TEMPERATURE

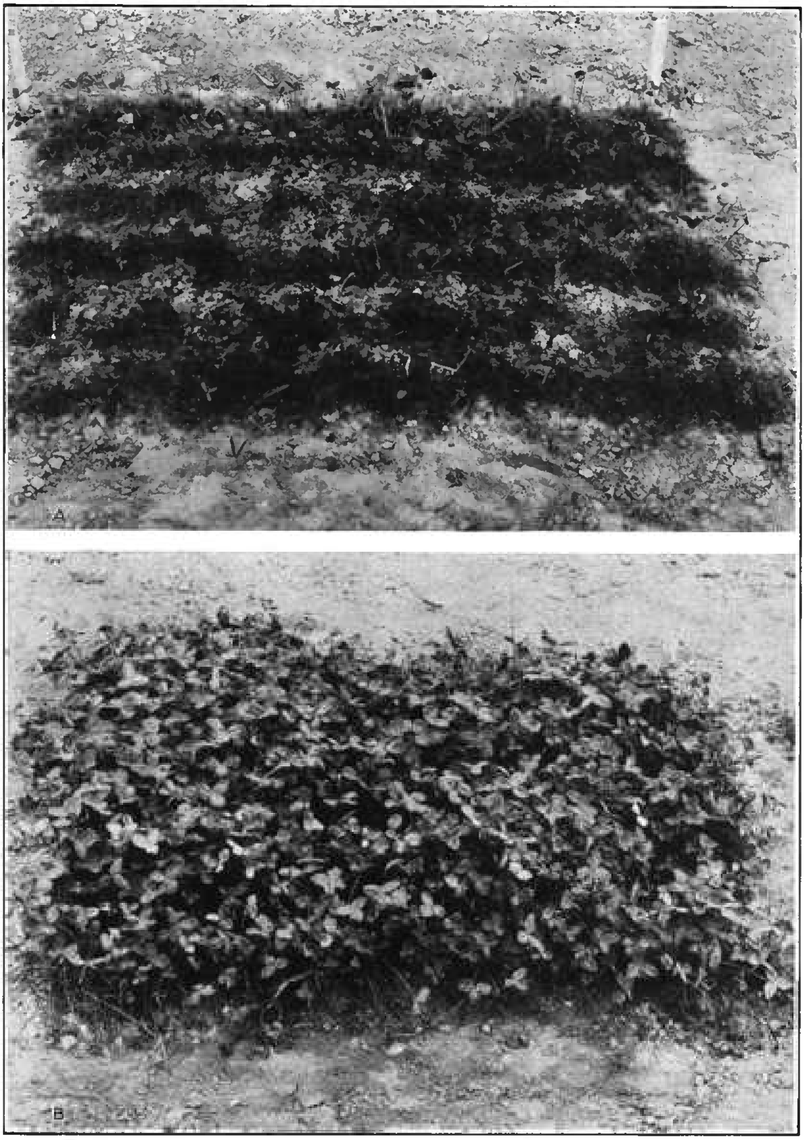
A, Potato-dextrose agar modified with HCl and NaOH to give the hydrogen-ion concentration indicated in the illustration. The cultures here shown were grown 10 days at 22° to 24° C. and show the wide range of acid or alkaline tolerance

B, Cultures on potato-dextrose agar grown eight days at constant temperatures as indicated. The optimum temperature for growth on this medium is 28° C. Plates maintained at temperatures 4° above and below the range here shown did not show any growth of the fungus. Note the abundance of sporulation at temperatures between 20° and 32°

EFFECTS OF AIR TEMPERATURE AND HUMIDITY ON ANTHRACNOSE INFECTION OF RED CLOVER

A, Effect of air temperature on anthracnose. These plants were inoculated at the same time with spores of *Colletotrichum trifolii* and placed in a moist chamber at 28° C. for two days. The pot on the left was then kept at 18° in an atmospheric humidity of 80 to 90 per cent saturation, the pot on the right being placed in another compartment at 24° with similar humidity. The photograph was taken six days after inoculation. No lesions were evident on the plants in the pot at the left when photographed, whereas in the other every leaf was affected. Plants kept at 28° continuously after inoculation showed injury similar to that at 24°

B, Effect of humidity on infection. Red-clover seedlings inoculated with *C. trifolii* and left in a moist chamber at 25° for 12 hours (left) and 24 hours (right). When removed from the moist chamber they were kept at 25° in an air humidity of 80 to 90 per cent. Note the freedom from disease of the plants which were exposed 12 hours as contrasted with the severe infection in plants exposed 24 hours in the moist chamber



VARIETAL RESISTANCE OF RED CLOVER TO ANTHRACNOSE

Plots of red clover were planted with Italian seed (A) and Tennessee anthracnose-resistant (B), from a series of plots planted at Arlington Experiment Farm, Rosslyn, Va., in 1924, to test the relative susceptibility to anthracnose of clover of different origins. In the case of the susceptible strain of Italian origin, the thick mat of dead plants indicates that a heavy stand was obtained. The Tennessee anthracnose-resistant strain showed a high degree of resistance, and although the disease was abundant, as is shown by the dead petioles in the front row, the plants were able to survive the attacks and completely recovered during the fall months site. From the practical standpoint this temperature relationship has an important bearing on control by means of the fall-seeding method.

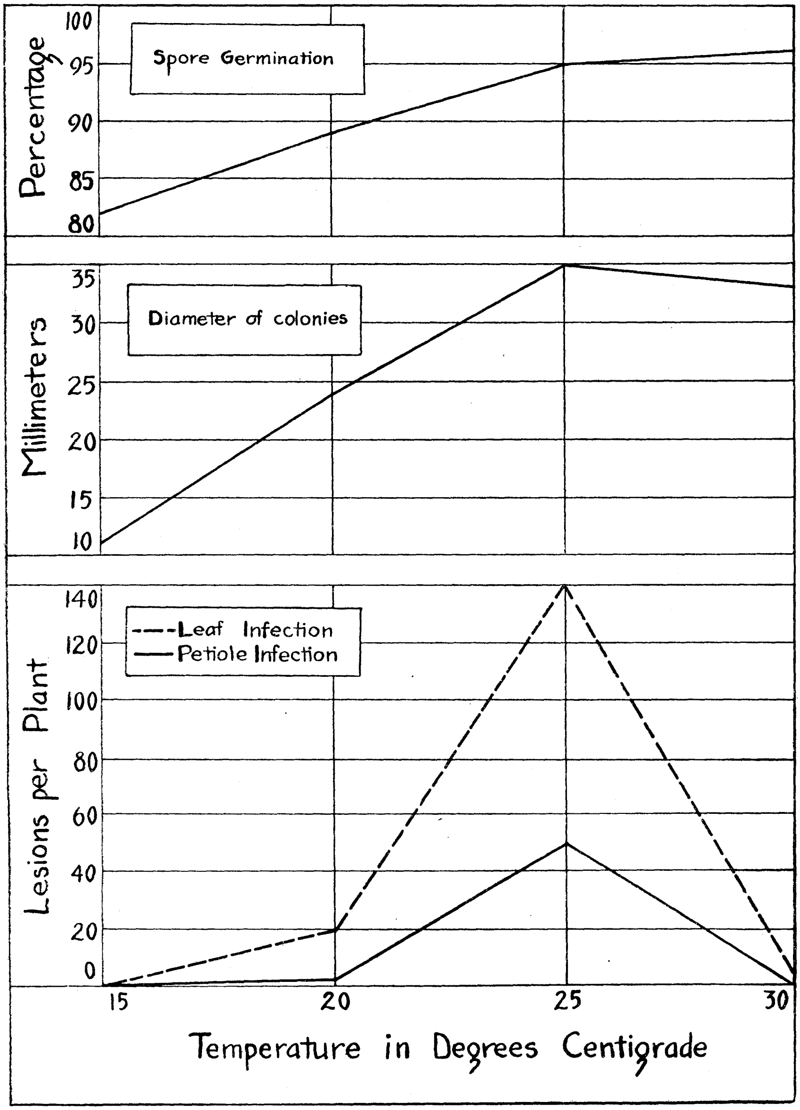


FIG. 4.—Effect of temperature on spore germination, growth on potato-dextrose agar, and infection of red-clover seedlings by *Colletotrichum trifolii.* These four curves represent results obtained in a typical series where spore germination, mycelial growth, and infection were studied simultaneously in the same constant-temperature moist chambers, the same suspension of spores being used for all.

MOISTURE

Although the disease frequently appeared to be worse during dry weather, it was apparent from field observations that high humidity was required for infection and that once infection occurred the disease could then continue to develop in dry periods. To test this influence of atmospheric moisture under controlled conditions, a number of pots containing five or six clover seedlings each were placed in one of the constant-temperature moist chambers. These plants were sprayed in the usual manner with a suspension of spores and then kept in a saturated atmosphere at 25° C. At regular intervals pots were transferred to another constant-temperature chamber where the humidity was maintained at about 90 per cent saturation. The number of lesions on leaf blades and petioles was recorded on the fourth day and daily thereafter. In Table 2, which contains the results from such a test, it will be seen that abundant moisture is required for more than 12 hours to produce infection. In this work the moisture condition most favorable for disease development appeared to be that in which the plants are covered with dew for 48 hours and thereafter kept in an atmosphere in which the leaves remained dry.

TABLE 2.—*Effect of atmospheric humidity on infection by Colletotrichum trifolii and the development of anthracnose on red-clover seedlings at 25° C.*

[The figures in the last six columns represent the number of distinct lesions evident on five plants which were first placed in a 25° C. moist chamber and removed at intervals to a 25° C. compartment with atmospheric humidity 80 to 90 per cent saturation]

Period in moist chamber	Number of leaves	Number of lesions					
		Four days after inoculation		Five days after inoculation		Six days after inoculation	
		On leaves	On petioles	On leaves	On petioles	On leaves	On petioles
0	38	0	0	0	0	0	0
⅛ day	42	0	0	0	0	0	0
¼ day	34	0	0	0	0	0	0
⅜ day	45	3	0	5	0	5	0
½ day	36	1	0	3	0	3	0
1 day	43	80	5	175	35	(1)	(1)
2 days	39	120	38	225	70	(1)	(1)
3 days	38	17	8	60	20	(1)	(1)
4 days	28	5	3	58	43	(1)	(1)
5 days	27			40	3	84	4
6 days	28					37	0

[1] The lesions had become so numerous and had coalesced to such an extent that counts were not attempted.

This dependence on moisture for infection partly explains the sporadic outbreaks of disease under field conditions and helps to explain the development of serious infections during some summers, whereas in other seasons the disease may be of scarcely any importance for many weeks, even though the temperature is favorable to its development. Periods of high humidity and heavy dew greatly stimulate spore production, and if there is also an occasional shower to distribute the conidia, the disease soon becomes widespread if the temperature is favorable. However, in the field it is frequently observed that after abundant spore production and a shower to scatter the inoculum there may be only a minor outbreak of the disease. This is probably due to the fact that wind or sunshine may dry the surface of the plants in time to avoid infection. The frequency of lesions on the stalks of the leaflets or at the margins of the leaves may no doubt be explained as due to the tendency of moisture to collect at such points and remain there longer than on other parts of the plant. The general distribution of lesions among the stems and leaves in thick stands is probably aided by the moister conditions found in heavy growth.

CONTROL

In a field crop such as clover many of our methods of plant-disease control are for the present obviously impracticable, even though they may be effective. Although spraying and dusting a clover crop is occasionally attempted, it is unlikely that such methods will ever be generally feasible against anthracnose. The same objection, to a somewhat lesser extent, applies also to the recommendation frequently made by pathologists that the disease be controlled by using a longer crop rotation. Since the value of this crop depends to a large extent on the place it occupies in a definite cropping system, application of such control measures is limited.

As the disease may be carried with the seed, various treatments might be used to prevent dissemination in this manner. Practically all tests which have been made to control clover anthracnose by seed disinfection have failed or at best have been only partially effective. This failure is probably due to the fact that clover is so generally distributed throughout the infested area that volunteer plants occur practically everywhere and undoubtedly serve to carry over the disease and infect new seedings, no matter how clean the seed may be. Also dead plants of the previous season or infected leaves from neighboring fields may start the disease in a new crop. For these reasons seed disinfection will probably seldom prove effective in controlling anthracnose.

A control method which is usually simple to use and at the same time effective under certain conditions is that of modifying the planting time to avoid the period of greatest severity of the disease. Since anthracnose is worst during the summer months, it frequently destroys or greatly reduces a clover stand which was planted in the spring of that year. If the seeding is made without a nurse crop during August, it is often possible to obtain an excellent stand which escapes the greater part of the period when the disease is most prevalent. Such a crop may develop rapidly during the cool fall weather when anthracnose is no longer dangerous, and by winter there is likely to be a much better stand than there would have been had the planting been made in spring and subjected to the thinning action of an attack during the summer. This method, which has been advocated for some time by the Tennessee Agricultural Experiment Station, has been of much value in checking the injuries produced by this disease in that State. In many cases, however, late summer or fall planting is impracticable. This method of control used alone is only partially effective, for though it avoids infection to some extent the first season, it insures nothing more than a single hay crop the following year, since a susceptible strain is likely to be destroyed soon after the hay is harvested. It appears that the time of planting as a means of checking this disease may be of considerable importance when used in conjunction with anthracnose-resistant strains of clover. However, the experimental work on this phase of control is at present not sufficiently extensive to justify any definite recommendation.

It is a common practice to plant red clover mixed with other crops, particularly grasses, such as timothy and redtop. When this method is used the damage caused by anthracnose is greatly reduced, probably because the clover plants are separated to some extent and the fungus is not likely to spread so readily. Furthermore, in such plantings a severe thinning of the clover stand is usually masked by the heavy growth of grass, so that the crop is not regarded as a failure. There are many times, however, when a pure stand of red clover is much more desirable than such a mixture, and for that reason application of this method of control is limited.

ANTHRACNOSE-RESISTANT CLOVER

By far the most effective and economical method for control of clover anthracnose is the use of species or strains resistant to its attack.

In 1905 (*14*) alsike clover, because of its decided resistance to this disease, was suggested as a substitute to replace medium red clover, which had for several years become increasingly difficult to grow throughout the middle section of Tennessee. Alsike, during recent years, has been gaining favor in certain sections, because it is also resistant to powdery mildew. However, this plant does not entirely fill the place of medium red clover in most of the clover belt.

Bain and Essary soon recognized the possibility of controlling the disease by means of resistant strains. As a result of their efforts (*2*) extending over a series of years, a strain of red clover was developed which was highly resistant to the anthracnose prevalent in that State. They pointed out (*7*) that their original selection probably was from a naturalized strain, the resistance of which was due to acclimatization. This stock soon became generally distributed throughout Tennessee, and wherever tested it was found to be effective in checking losses caused by this disease. In spite of the fact that no reselections have been made in recent years, clover which can be traced back to that particular strain is invariably outstanding in vigor and resistance to *Colletotrichum trifolii*. It has been observed that this Tennessee strain, when planted in Europe and the Northern States, is apt to be seriously injured by anthracnose. In such cases the parasite has been *Gloeosporium caulivorum* rather than the fungus against which the strain was developed.

In 1914 (*3*) it was pointed out that resistance in the Tennessee strain was probably due to a difference in the chemicals of the cell sap. However, it is apparent that the difference between susceptible and resistant plants is not simply a chemical influence on the spores, as was indicated by those experiments. In artificial inoculations and also under field conditions spore germination occurs on resistant plants, and abundant infection may develop. In resistant strains the progress of the fungus is less rapid, and the total injury to the plant is therefore much less permanent. This contrast is most striking when infection is on the lower part of the stems or petioles, for on susceptible strains a rapid spread of the fungus soon involves the crown and destroys the plant. Whether this development within the tissue is wholly or in part due to variations in the chemical composition of the cell sap has not been determined.

Varietal differences in the degree of resistance to *Colletotrichum trifolii* have been observed also in alfalfa at the Mississippi station (*15*).

FOREIGN COMPARED WITH HOME-GROWN SEED

The results of field comparisons of red-clover seed of different origin, both from foreign countries and from various sections in this country, have been reported previously (*12*, *18*). An example of such a test is shown in Plate 7, which shows two typical plots taken from a series grown under the same environmental conditions on the Arlington farm, planted in the spring of 1923 and photographed in August of that year. All started evenly with a good stand and were inoculated by scattering over them dead plants collected from a clover field which had been completely destroyed by anthracnose during the previous season. The disease was abundant in the plot of the resistant strain (Tennessee anthracnose resistant) but killed very few plants, whereas in the plot of susceptible clover (Italian) scarcely any withstood the attack.

These observations have been further checked by greenhouse inoculations under controlled conditions which eliminated the possibility of confusion with other diseases or differences due to dissimilar response to extreme climatic conditions. Certain of the latter affect the Italian clover much more adversely than they do the Tennessee strain. The results obtained from the greenhouse tests correspond closely to those from the field plantings.

As a general rule it may be stated that imported red-clover seed, especially that originating in Italy, is much more susceptible to the disease than is our native-grown stock produced for several generations in regions where this anthracnose is common. Even in the absence of any widespread infection, the susceptible imported strains are ordinarily weaker and much less desirable than locally grown stock, but during a severe attack of anthracnose these differences are much more striking, for at such times plantings made with Italian and similar seed usually are completely destroyed. Unfortunately from the standpoint of control, no simple rule based on geographical origin of seed can be applied as a guide in the purchase of resistant stock. Red-clover seed originating in certain parts of Europe is invariably extremely susceptible to *Colletotrichum trifolii*, but that from other sections has regularly shown a fair degree of resistance. Likewise, there is a great difference in this respect among our American-grown seed. That obtained from Tennessee or other Southern States is not necessarily resistant. If it is from a stock that has been grown in an anthracnose-infested area for several generations it is likely to be much more resistant than most of that grown in the Northern States and Canada. Seed from the Northwestern States has invariably proved to be dangerously susceptible to anthracnose.

THE DEVELOPMENT OF RESISTANT CLOVER

The extreme resistance to *Colletotrichum trifolii* still manifest in the Tennessee strain indicates that when once secured this character is likely to be maintained, provided the stock is grown regularly in a region where the disease is common. Whether this resistance would be preserved if the stock were grown for several generations in a section where the disease does not occur has not been determined. It seems likely, however, that loss of this character would be a relatively slow process anywhere in this country.

The early work in Tennessee demonstrated that it was entirely practicable to develop a strain of anthracnose-resistant red clover by means of artificial inoculation and continued mass selections. By further careful breeding it is probable that this character of resistance could be further developed and that a much more valuable strain of clover might be produced. At the present time, however, no further work is being done toward such improvement.

Further development of the anthracnose-resistant strains now available apparently will depend on the process of natural selection, for some time at least. Home-grown red-clover seed from a stock which has been developed in a section of the country where this particular anthracnose is regularly severe is, as a general rule, much more resistant than strains originating in localities where the disease is unimportant. In territories where the disease is severe the customary crop practices and the characteristics of the disease are such that a process of natural selection is likely to become gradually effective in increasing the resistance to anthracnose. When the seeding is made in the spring the young stand is exposed during the summer to the thinning action of the disease whenever it is active. Thus the most susceptible individuals are usually eliminated during the seedling year. The following season the first growth is, as a rule, cut for hay, and what seed is harvested is in most cases obtained from the second crop. This means that the seed-producing plants are again exposed to the unfavorable summer months when anthracnose is likely to reduce further the number of plants. As those more resistant approach the blossoming stage they are exposed to stem infection, which is especially common at a point just below the flower head. As a result of this type of infection great numbers of heads are cut off by the disease and fail to mature. Therefore, in fields which are badly infected during two summers, only the most resistant plants are able to produce seed. Unfortunately for this type of selection, anthracnose is apt to occur in patches over the field, so that seed from such resistant plants is frequently diluted with some from more susceptible individuals growing in a section of the field where the disease was not so severe.

This process of natural selection undoubtedly has produced the various strains of red clover found in the southern section of our clover belt, which exhibit a considerable degree of resistance to *Colletotrichum trifolii* when compared with imported or northern-grown red clover.

Since losses due to anthracnose can be so effectively reduced by using resistant strains, and since these strains can be improved in any section where infection is commonly severe, the use of home-grown seed should be encouraged as the most effective means for checking the disease in such regions. The prejudice against "diseased seed" in this case is unfounded. In spite of the customary advice to use "disease-free seed," farmers should be encouraged to harvest as much as possible from stands greatly reduced by anthracnose, for such seed will eventually produce the best crops.

SUMMARY

An investigation of diseases as a factor in "clover failure" led to a study of the two principal anthracnose-producing fungi, *Colletotrichum trifolii* and *Gloeosporium caulivorum*. This bulletin deals primarily with *C. trifolii*, which occurs most injuriously in the southern half of the clover belt of the United States.

This disease was first reported in Tennessee in 1905 and has since been found generally distributed throughout the eastern and mid-western clover belt. Only once has it been noted outside of North America.

The host range includes certain species of Trifolium, Medicago, Melilotus, and probably others; but many legumes are immune.

Detailed description is given of symptom on leaves, stalks of leaflets, petioles, stems, crowns, and roots. Symptoms of *C. trifolii* and *G. caulivorum* on red clover are similar, microscopic examination being usually necessary for positive identification.

The fungus penetrates cortex, bundles, and pith, causing complete collapse of the tissue. Infection of leaves, petioles, or stems may greatly lessen the yield of hay, but the more important type of injury is crown or root infection which kills the plants and thereby greatly reduces the value of the crop from the standpoint of soil improvement.

Attention is called to the occasional confusion of *C. trifolii* with *G. caulivorum*, but differences in spore characters, setae, and host range fully justify separation of these fungi. Anthracnose of clover has been found to be produced by other forms of Colletotrichum which appear to be sufficiently distinct for specific rank.

C. trifolii is isolated on most of the common culture media. Spores germinate well in distilled water, but do better in diluted juices or decoctions from the tissues of host plants.

Cultural characteristics of the fungus are described. It grows and sporulates readily on a large number of agar preparations as well as on cooked plant tissue. A study of the relationship of temperature to growth on artificial media showed that the minimum is about 10° C., optimum approximately 28°, and maximum slightly above 35°. Sporulation is slight below 16°.

Growth on agar in diffused daylight was practically the same as that in constant darkness at the same temperature. Relatively high humidity is required for spore germination.

On potato-dextrose agar the fungus was able to grow over a hydrogen-ion range of 3 to 9.5. The optimum was found to be between 3.7 and 6.6. Sporulation was greatly reduced in the more acid agar.

Its pathogenicity has been demonstrated on *Trifolium pratense*, *T. pratense perenne*, *T. incarnatum*, *Medicago sativa*, *M. hispida*, and *Melilotus alba*. Any of these hosts are readily penetrated directly by the fungus.

C. trifolii is favored by periods of hot weather which are unfavorable to red clover, so that the disease causes most damage during the summer months. In periods of high humidity conidia are produced in large quantities. No other form of spore has been observed.

The fungus retains its viability on dried material for long periods and may survive unfavorable conditions on infected tissue of either living or dead plants.

Dissemination of the fungus may be accomplished by wind-blown dried infected leaves or stems. Local spore distribution is probably largely by means of spattering rain. Insects and animals may also carry the conidia.

Infection of leaves and stems usually takes place directly through the uninjured epidermis. Crown or root infection is frequently greatly aided by insect injuries. The period of incubation under favorable conditions of temperature and moisture is three to five days.

Primary infection may come from diseased parts of plants carried with seed from such portions blown from near-by fields or from diseased plant refuse left from the previous season.

Infection experiments conducted at constant air temperatures showed that temperature is an important factor in infection and later development of the disease. The optimum for disease development is approximately the same as that for growth on potato-dextrose agar—about 28° C. This temperature response largely accounts for the geographical distribution and seasonal developments of the disease.

The fungus requires abundant atmospheric moisture for most rapid spore production and infection. After penetration occurs the disease becomes more active and injurious in a drier atmosphere.

Various methods of control have been suggested, including spraying or dusting, crop rotation, seed disinfection, fall planting, and mixtures with grass. Such methods are regarded as decidedly limited in their application to clover-anthracnose control.

Control by means of resistant varieties offers the most promise. The Tennessee anthracnose-resistant strain of red clover still shows decided resistance in spite of the fact that no recent selections have been made. Home-grown clover seed from stock produced in regions where the disease is regularly severe is in general much more resistant to anthracnose than are the imported or northern-grown strains. Because of the common practices in clover culture and the characteristics of the disease the process of natural selection must play an important part in increasing the degree of resistance in clover grown continuously in an anthracnose-infested region.

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